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Original Article

Myeloid-related protein-8/14 and C-reactive protein in individuals evaluated for obstructive sleep apnea

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ABSTRACT

Background: Obstructive sleep apnea (OSA) and obesity are often present concomitantly. Their potential contribution to inflammation remains an ongoing debate. The objectives of this study were to investigate whether variables of sleep-disordered breathing are associated with levels of myeloid-related protein-8/14 (MRP-8/14) or C-reactive protein (CRP), and to characterize how adiposity interacts with these associations in individuals evaluated for possible OSA.

Methods: Consecutive individuals referred to Lovisenberg Diakonale Hospital's sleep laboratory between 1st October 2009 and 1st March 2010 were included. We characterized the biomarker distribution sampled the morning after sleep and related these to clinical characteristics and variables recorded during polygraphy or polysomnography.

Results: Of the total study population of 222 individuals, 161 (72.5%) were diagnosed with OSA (apnea–hypopnea index (AHI) $\geqslant$5/h). In baseline models (multiple median regression adjusted for age and sex), AHI was independently associated with MRP-8/14 ($P = 0.025$) and CRP ($P < 0.001$). The associations were attenuated after the addition of body mass index (BMI), but remained statistically significant for CRP ($P = 0.025$). However, in final models adjusted for additional factors (systolic blood pressure, cholesterol:high-density lipoprotein ratio, glycosylated haemoglobin, smoking, and cardiovascular disease), only average oxygen saturation for MRP-8/14 ($P = 0.028$) and oxygen desaturation index (ODI) for CRP ($P = 0.037$) remained independent predictors of inflammation, whereas AHI lost its predictive value (MRP-8/14; $P = 0.30$ and CRP; $P = 0.092$). The association between several variables of sleep-disordered breathing and inflammation were stronger in individuals with a higher BMI (P for interaction <0.05 for AHI, nadir oxygen saturation, and time <90% oxygen saturation).

Conclusions: No definitive indication of independent immunological activity resulting from apneas and hypopneas was found in final models adjusted for other factors associated with inflammation, whereas average oxygen saturation for MRP-8/14 and ODI for CRP remained statistically significant predictors. Interactions were observed between BMI and several variables of sleep-disordered breathing on MRP-8/14 and CRP levels.

1. Introduction

Obstructive sleep apnea (OSA) is a widespread condition characterized by repetitive abnormal breathing giving rise to disrupted ventilation during sleep [1]. Patients with OSA are at increased risk of cardiovascular disease (CVD) [2]. The specific pathophysiological processes and pathways involved remain an area of intense research [3]. Knowledge about these mechanisms may ultimately allow for development of preventive and therapeutic strategies for OSA patients, and could also enhance our comprehension of the pathophysiological basis of CVD in general. Systemic inflammation has been implicated in CVD in other populations

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and represents a plausible contributing factor for development of cardiovascular complications in OSA [4,5]. In spite of numerous studies, the specific role of inflammation in OSA remains obscure.

Myeloid-related protein-8/14 (MRP-8/14) is a heterodimer with proinflammatory characteristics expressed by monocytes, neutrophils, and platelets. It is a complex of two calcium-binding proteins that possess intra- and extracellular activity (MRP-8 (S100A8) and MRP-14 (S100A9)). MRP-8 and MRP-14 are displayed by monocytes upon interaction with activated endothelium and reflect phagocyte stimulation [6,7]. They are members of the S100 family of proteins involved in the inflammatory response through, in part, activation of the receptor for advanced glycation end-products found to be elevated in OSA [8-12]. In animal studies MRP-8/14 is critical for the biological response to vascular injury [13]. Circulating MRP-8/14 has been shown to be associated with inflammatory disorders and myocardial infarction and to be an independent predictor of cardiovascular events in healthy subjects and patients with acute coronary syndrome [7,14–19]. It has been demonstrated that children with OSA have elevated levels of MRP-8/14 [20,21].

C-reactive protein (CRP) is a well-established inflammatory marker secreted through different mechanisms than MRP-8/14. This enzyme has been thought to be synthesized by hepatocytes in response to factors produced by adipocytes and inflammatory cells. Interestingly, there have been reports of CRP expression in other locations including coronary artery smooth muscle and adipose tissue [22–24]. CRP is a predictor of cardiovascular events in apparently healthy individuals [4,5]. It has been studied in OSA with conflicting results conceivably pertaining to variable sample size and type as well as degree of confounding factors [25–28]. Recently, a large meta-analysis showed that several inflammatory biomarkers including CRP were higher in OSA when compared to controls, and another meta-analysis found evidence that treatment with continuous positive airway pressure could partially suppress inflammation [29,30].

In this study, we wanted to test the hypothesis that the stress induced by sleep-disordered breathing will stimulate an inflammatory response detectable by MRP-8/14 or CRP. Thus, our objectives were to investigate whether variables of sleep-disordered breathing are associated with levels of MRP-8/14 or CRP and to characterize how adiposity interacts with these potential associations in individuals evaluated for possible OSA.

2. Methods

2.1. Study population

This was a single-center, cross-sectional study in which all consecutive individuals referred by general practitioners or Ear–Nose–Throat specialists to the sleep laboratory at Lovisenberg Diakonale Hospital for evaluation of possible OSA from October 2009 through February 2010 were considered. Participants were excluded if they had previously been diagnosed with OSA, if the sleep study indicated another type of sleep-disordered breathing than OSA, or if there was no serum sampled for biobank (due to technical difficulties or if they did not consent).

2.2. Clinical characteristics

Comorbidity data (hypertension, heart disease, stroke/transient ischemic attack, diabetes, and smoking status) were extracted from a questionnaire and from each patient's medical record. Heart disease was defined as coronary heart disease, heart failure, or atrial fibrillation. CVD was defined as heart disease and/or stroke/transient ischemic attack. Physical indices were recorded prior to the sleep study. Blood pressure (BP) measurements were performed in agreement with standard techniques by sphygmomanometer on the upper arm. Body mass index (BMI; kg/m^2) was calculated.

2.3. Sleep study

Participants were examined with overnight in-home polygraphy (PG) or in-hospital polysomnography (PSG). The in-home, unattended PG was performed with a standard 10-channel cardiorespiratory recording device (Embletta Portable Diagnostic System (PDS), ResMed, Høvik, Norway) or a 12-channel monitor with a nasopharyngeal/oesophageal catheter measuring flow and pressure in the upper airways and indirectly the intrathoracic pressure (Reggie, Camtech, Høvik, Norway). The attended, in-hospital PSG (Embla S4500, ResMed) included a six-channel electroencephalogram, a two-channel electrooculogram, submental electromyogram (EMG), thoracic and abdominal movements (respiratory inductance plethysmography), air flow (nasal air pressure catheter), pulse oximetry, EMG from both legs, body position, and a three-channel electrocardiogram.

The data from the sleep study was scored by one single qualified sleeping disorder specialist not involved in the study. A modified version of the 2007 American Academy of Sleep Medicine criteria for scoring respiratory events was used [31]. Apnea was measured via transformed airflow signals from nasal pressure and defined as cessation of airflow $\geqslant$10 s. Hypopnea was defined as a 50% reduction in airflow with either a $\geqslant$3% oxyhemoglobin desaturation, or an arousal or a presumed arousal (an increase of 10% in heart rate). The apnea–hypopnea index (AHI) was calculated based on the total number of events per hour of total recording (PG) or sleep (PSG) time (movement time omitted). Oxygen desaturation index (ODI) was estimated by the average number of desaturations of $\geqslant$3% per hour. OSA was defined as AHI $\geqslant$5 (symptoms of sleepiness were not considered).

2.4. Blood sample handling and analyses

Fasting blood samples were obtained the morning after the sleep study and directly processed for analysis of glycosylated haemoglobin (HbA1c) and a complete lipid profile on automated platforms. Serum aliquots for MRP-8/14 were stored at −20 °C, and within the same week transferred to a −80 °C freezer to allow for batch analysis using a commercially available MRP8/14 enzyme-linked immunosorbent kit (Bühlmann Laboratories AG, Schönenbuch, Switzerland). According to the manufacturer this assay has an analytical range of 0.4–24 μg/mL, an intra-assay coefficient of variation (CV) of 4.3% (20 pairs of values from seven different serum samples each obtained in a single run), and an inter-assay CV of 5.8% (four samples each in 20 different runs). For a normal population their stated expected median value for serum is 1.14 μg/mL with a 95th percentile at 2.9 μg/mL (considered as the upper limit of the normal reference range). These values were estimated by the manufacturer from apparently healthy male and female blood donors aged 18–70 years. All samples for MRP-8/14 were analysed in duplicates by one experienced biomedical laboratory scientist blinded to study data, with a reported average CV of 4.0%. Readings <0.4 μg/mL were interpreted as undetectable. An immunoturbidimetric Tina-quant C-reactive Protein Gen. 3 (CRPL3) assay on an automated Modular Analytics P platform by Roche Diagnostics (Roche, Basel, Switzerland) was used for quantification of CRP from fresh serum (single measurements on the day of collection). According to the packet insert this assay has an analytical range of 0.3–350 mg/L, with a designated normal reference range <5 mg/L [32]. Our laboratory considered concentrations <1.0 mg/L as undetectable, and reported a between-run CV of 2.3% at 4.3 mg/L and 2.9% at 73.2 mg/L.

2.5. Ethical considerations

The study was conducted according to the principles of the Declaration of Helsinki. It was approved by our hospital's local ethics committee, the privacy protection act supervisor, the Norwegian data inspectorate and the regional ethics committee. All participants gave written informed consent.

2.6. Statistical analysis

Continuous variables were compared between groups with the two-sample *t*-test (if approximately normally distributed) or the Mann–Whitney *U*-test (if markedly skewed). Categorical variables were compared using the Fisher mid-*P*-test [33]. Spearman rank correlation was used to assess simple (univariate) associations of continuous variables with MRP-8/14 or CRP. Multivariable median regression analyses were performed to investigate simultaneous associations of several predictors of MRP-8/14 or CRP. Median regression is similar to linear regression. Both models describe the association(s) between a continuous dependent variable and one or more explanatory variables. Linear regression enables inference about the mean of the dependent variable, whereas median regression enables inference about the median. Model-building techniques and general regression features, such as the ability to study interactions and non-linear effects, are available for median regression. Three models were designed to evaluate the characteristics of the potential associations between variables of sleep-disordered breathing and inflammation: a baseline model to account for age and sex; a BMI model to account for age, sex and BMI; and a final model to account for age, sex, BMI, systolic BP, cholesterol:high-density lipoprotein (HDL) ratio, HbA1c, smoking, and CVD. The potential interaction between BMI and variables of sleep-disordered breathing on inflammation were probed by addition of an interaction term (variable of sleep-disordered breathing × BMI) to the final models. Undetectable values of MRP-8/14 (<0.4) and CRP (<1.0) were imputed using uniformly distributed random values on the intervals 0.0–0.4 (MRP-8/14) and 0.0–1.0 (CRP). This imputation method was preferred after no noteworthy disagreements were observed when compared with sensitivity analyses using extreme case scenarios. The statistical analyses were performed with PASW Statistics 18 (IBM SPSS Inc., Chicago, IL, USA) and STATA version 12.1 (StataCorp LP, College Station, TX, USA). $P < 0.05$ was regarded statistically significant, and all hypothesis testing was two-tailed.

3. Results

3.1. Demographics

In all, 222 individuals were included in the study, of which 64 (28.8%) were females and 158 (71.2%) were males; 161 (72.5%) were diagnosed with OSA (AHI ⩾5), and these were older with a higher BMI. A larger proportion had comorbidities such as hypertension and CVD when compared to individuals without OSA. The distribution of sexes was roughly equal. Table 1 shows baseline characteristics of the two groups.

3.2. MRP-8/14 and CRP values

Overall median (quartile 1–3) values were 2.4 (1.6–3.5) μg/mL for MRP-8/14 and 1.0 (<1.0–3.0) mg/L for CRP. No samples were above the analytical range for either assay. 76 samples (34.2%) were above the MRP-8/14 normal reference range at >2.9 μg/mL and 3 (1.4%) measured <0.4 μg/mL, whereas 41 samples (18.5%) were above the CRP normal reference range at ⩾5 mg/L and 84 (37.8%) measured <1.0 mg/L. There was a significant correlation between the two biomarkers (Spearman's ρ, 0.29; 95% confidence interval (CI), 0.17–0.41; $P < 0.001$). Table 2 shows simple associations of MRP-8/14 or CRP to continuous variables. MRP-8/14 was negatively correlated with age. CRP was most strongly correlated with BMI (Spearman's ρ, 0.49; 95% CI, 0.38–0.58; $P < 0.001$) and displayed associations with all variables of sleep-disordered breathing ($P < 0.001$ for all).

3.3. Apnea–hypopnea index and inflammation

In baseline models (multiple median regression adjusted for age and sex), AHI was an independent predictor of MRP-8/14 ($P = 0.025$)

Table 1
Baseline characteristics of 222 individuals investigated for possible obstructive sleep apnea.

Variable	AHI <5 ($n = 61$)	AHI ⩾5 ($n = 161$)	*P*
Age (years)	43 ± 11	50 ± 13	<0.001
Male sex	39 (63.9%)	119 (73.9%)	0.16
AHI (events/h)	2.7 (1.0–3.4)	17.4 (8.6–31.4)	<0.001
ODI (events/h)	5.2 (2.8–8.5)	20.6 (12.3–37.5)	<0.001
Average oxygen saturation (%)	95.4 (94.1–96.1)	93.9 (92.8–95.1)	<0.001
Nadir oxygen saturation (%)	90 (88–92)	84 (80–87)	<0.001
Time <90% oxygen saturation (min)	0.2 (0–1.2)	7.1 (1.2–22.8)	<0.001
Heart rate (beats/min)	62 ± 12	65 ± 11	0.14
BMI (kg/m^2)	26.8 ± 3.8	30.2 ± 5.5	<0.001
Systolic BP (mmHg)	128 ± 15	136 ± 16	<0.001
Diastolic BP (mmHg)	84 ± 11	88 ± 12	0.076
Current smoker	16 (26.7%)	29 (19.1%)	0.23
CVD	4 (6.6%)	27 (16.8%)	0.041
Hypertension	15 (24.6%)	65 (40.4%)	0.024
Diabetes	3 (4.9%)	12 (7.5%)	0.66
HbA1c (%)	5.5 ± 0.4	5.8 ± 0.7	<0.001
Cholesterol (mmol/L)	5.2 ± 0.9	5.4 ± 1.1	0.30
LDL (mmol/L)	3.2 ± 0.8	3.4 ± 1.0	0.21
HDL (mmol/L)	1.4 ± 0.4	1.2 ± 0.4	0.014
Triglycerides (mmol/L)	1.5 ± 0.9	1.9 ± 2.3	0.18
Cholesterol:HDL ratio	4.0 ± 1.2	4.7 ± 1.6	0.002
MRP-8/14 (μg/mL)	2.6 (1.6–3.7)	2.3 (1.6–3.2)	0.259
CRP (mg/L)	<1.0 (<1.0–2.0)	2.0 (<1.0–4.0)	0.001

AHI, apnea–hypopnea index; ODI, oxygen desaturation index; BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; HbA1c, glycosylated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MRP-8/14, myeloid-related protein-8/14; CRP, C-reactive protein.
Data presented as mean ± standard deviation, median (quartiles 1–3) or number (%).

Table 2
Spearman correlation coefficient (95% confidence interval) of inflammatory biomarkers myeloid-related protein-8/14 (MRP-8/14) and C-reactive protein.

Variable	MRP-8/14	*P*	C-reactive protein	*P*
Age	−0.14 (−0.27 to −0.012)	0.032	0.099 (−0.034 to 0.23)	0.14
BMI	0.14 (0.005 to 0.26)	0.043	0.49 (0.38 to 0.58)	<0.001
Systolic BP	0.024 (−0.12 to 0.17)	0.74	0.19 (0.053 to 0.33)	0.008
AHI	−0.014 (−0.15 to 0.12)	0.84	0.31 (0.18 to 0.42)	<0.001
ODI	0.044 (−0.088 to 0.18)	0.51	0.39 (0.28 to 0.50)	<0.001
Average oxygen saturation	−0.083 (−0.21 to 0.050)	0.22	−0.27 (−0.39 to −0.15)	<0.001
Nadir oxygen saturation	0.021 (−0.11 to 0.15)	0.75	−0.34 (−0.46 to −0.22)	<0.001
Time <90% oxygen saturation	−0.010 (−0.14 to 0.12)	0.88	0.27 (0.14 to 0.38)	<0.001
HbA1c	0.002 (−0.13 to 0.13)	0.97	0.14 (0.011 to 0.27)	0.033
Cholesterol	−0.083 (−0.21 to 0.049)	0.22	0.070 (−0.062 to 0.20)	0.30
LDL	−0.034 (−0.17 to 0.99)	0.62	0.14 (0.002 to 0.26)	0.047
HDL	−0.16 (−0.29 to −0.032)	0.015	−0.21 (−0.33 to −0.083)	0.002
Cholesterol:HDL ratio	0.105 (−0.027 to 0.23)	0.120	0.23 (0.11 to 0.35)	<0.001
Triglycerides	0.083 (−0.050 to 0.21)	0.22	0.19 (0.059 to 0.31)	0.005

BMI, body mass index; BP, blood pressure; AHI, apnea–hypopnea index; ODI, oxygen desaturation index; HbA1c, glycosylated haemoglobin; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

and CRP ($P < 0.001$) concentrations. The associations were attenuated after the addition of BMI, but remained statistically significant for CRP ($P = 0.025$). After adjustment for additional factors in the final models (systolic BP, cholesterol:HDL ratio, HbA1c, smoking, and CVD), AHI did not remain a statistically significant predictor (MRP-8/14; $P = 0.30$ and CRP; $P = 0.092$) (Tables 3 and 4). For both biomarkers, the association between AHI and inflammation was stronger in individuals with a higher BMI (P for interaction = 0.039 for CRP and 0.024 for MRP-8/14) (Table 5 and Fig. 1).

3.4. Oxygen variables and inflammation

Oxygen desaturation index ($P = 0.023$), average oxygen saturation ($P = 0.004$), and nadir oxygen saturation ($P = 0.027$) were associated with MRP-8/14 whereas all four hypoxia indices ($P \leqslant 0.001$) were associated with CRP in baseline models. After addition of BMI the associations attenuated, but ODI remained a statistically significant predictor of CRP ($P = 0.015$). In the final models, average oxygen saturation for MRP-8/14 ($P = 0.028$) and ODI for CRP ($P = 0.037$) were observed to be statistically significant associations (Tables 3 and 4). Similarly with respect to AHI, several oxygen variables displayed interactions with BMI for both markers of inflammation (Table 5 and Fig. 1).

4. Discussion

This study evaluated the degree of inflammation measured by MRP-8/14 and CRP in individuals undergoing a clinical work-up for possible OSA. An association was identified between a majority of the variables of sleep-disordered breathing and inflammatory biomarker levels independent of age and sex. The associations attenuated after the addition of BMI, but remained statistically significant for AHI and ODI to CRP. After adjustment for additional factors associated with inflammation, independent associations

Table 3
Median regression models of myeloid-related protein-8/14 fitted on variables of sleep-disordered breathing using multivariate models.

Explanatory variables	Baseline model[a]		BMI model[b]		Final model[c]	
	Coefficient (95% CI)	*P*	Coefficient (95% CI)	*P*	Coefficient (95% CI)	*P*
AHI (events/h)	0.014 (0.001 to 0.026)	0.036	0.005 (−0.009 to 0.018)	0.49	0.009 (−0.008 to 0.027)	0.30
ODI (events/h)	0.014 (0.002 to 0.026)	0.023	0.003 (−0.010 to 0.016)	0.64	0.007 (−0.010 to 0.023)	0.44
Average oxygen saturation (%)	−0.176 (−0.297 to −0.055)	0.004	−0.116 (−0.251 to 0.018)	0.089	−0.193 (−0.364 to −0.021)	0.028
Nadir oxygen saturation (%)	−0.037 (−0.070 to −0.004)	0.027	−0.023 (−0.060 to 0.014)	0.23	−0.027 (−0.075 to 0.021)	0.27
Time <90% oxygen saturation (min)	0.002 (−0.002 to 0.006)	0.29	0.001 (−0.003 to 0.005)	0.72	0.001 (−0.005 to 0.007)	0.71

BMI, body mass index; CI, confidence interval; AHI, apnea–hypopnea index; ODI, oxygen desaturation index.
The coefficients are the estimated median difference in myeloid-related protein-8/14 per one unit increase of the explanatory variable.
[a] Adjusted for age and sex.
[b] Adjusted for age, sex, and BMI.
[c] Adjusted for age, sex, BMI, systolic blood pressure, cholesterol:high-density lipoprotein ratio, glycosylated hemoglobin, smoking, and cardiovascular disease.

Table 4
Median regression models of C-reactive protein fitted on variables of sleep-disordered breathing using multivariate models.

Explanatory variables	Baseline model[a]		BMI model[b]		Final model[c]	
	Coefficient (95% CI)	*P*	Coefficient (95% CI)	*P*	Coefficient (95% CI)	*P*
AHI (events/h)	0.045 (0.028 to 0.062)	<0.001	0.023 (0.003 to 0.043)	0.025	0.017 (−0.003 to 0.037)	0.092
ODI (events/h)	0.049 (0.031 to 0.066)	<0.001	0.025 (0.005 to 0.044)	0.015	0.021 (0.001 to 0.040)	0.037
Average oxygen saturation (%)	−0.327 (−0.491 to −0.163)	<0.001	−0.084 (−0.289 to 0.120)	0.42	0.024 (−0.174 to 0.222)	0.81
Nadir oxygen saturation (%)	−0.124 (−0.176 to −0.071)	<0.001	−0.045 (−0.098 to 0.007)	0.092	−0.038 (−0.094 to 0.019)	0.19
Time <90% oxygen saturation (min)	0.010 (0.004 to 0.016)	0.001	−0.002 (−0.008 to 0.005)	0.63	−0.003 (−0.010 to 0.003)	0.31

BMI, body mass index; CI, confidence interval; AHI, apnea–hypopnea index; ODI, oxygen desaturation index.
The coefficients are the estimated median difference in C-reactive protein per one unit increase of the explanatory variable.
[a] Adjusted for age and sex.
[b] Adjusted for age, sex, and BMI.
[c] Adjusted for age, sex, BMI, systolic blood pressure, cholesterol:high-density lipoprotein ratio, glycosylated hemoglobin, smoking, and cardiovascular disease.

Table 5
Median regression models of MRP-8/14 or CRP fitted on variables of sleep-disordered breathing × BMI using multivariate models with interaction terms.

Interaction term [× BMI (kg/m^2)]	MRP-8/14 model[a]		CRP model[a]	
	Coefficient (95% CI)	P[b]	Coefficient (95% CI)	P[b]
AHI (events/h)	0.003 (0.000 to 0.006)	0.024	0.004 (0.000 to 0.008)	0.039
ODI (events/h)	0.002 (−0.001 to 0.004)	0.18	0.003 (−0.000 to 0.006)	0.056
Average oxygen saturation (%)	−0.014 (−0.034 to 0.006)	0.18	−0.034 (−0.060 to −0.007)	0.012
Nadir oxygen saturation (%)	−0.010 (−0.017 to −0.003)	0.005	−0.013 (−0.021 to −0.005)	0.001
Time <90% oxygen saturation (min)	0.001 (0.000 to 0.002)	0.017	0.002 (0.000 to 0.003)	0.012

MRP-8/14, myeloid-related protein-8/14; CRP, C-reactive protein; BMI, body mass index; AHI, apnea–hypopnea index; ODI, oxygen desaturation index.
The coefficients are the estimated median difference in MRP-8/14 or CRP per one unit increase of the explanatory variable.
[a] Adjusted for age, sex, systolic blood pressure, cholesterol:high-density lipoprotein ratio, glycosylated hemoglobin, smoking, cardiovascular disease, and main effects of the variables involved in the interaction term.
[b] Interaction.

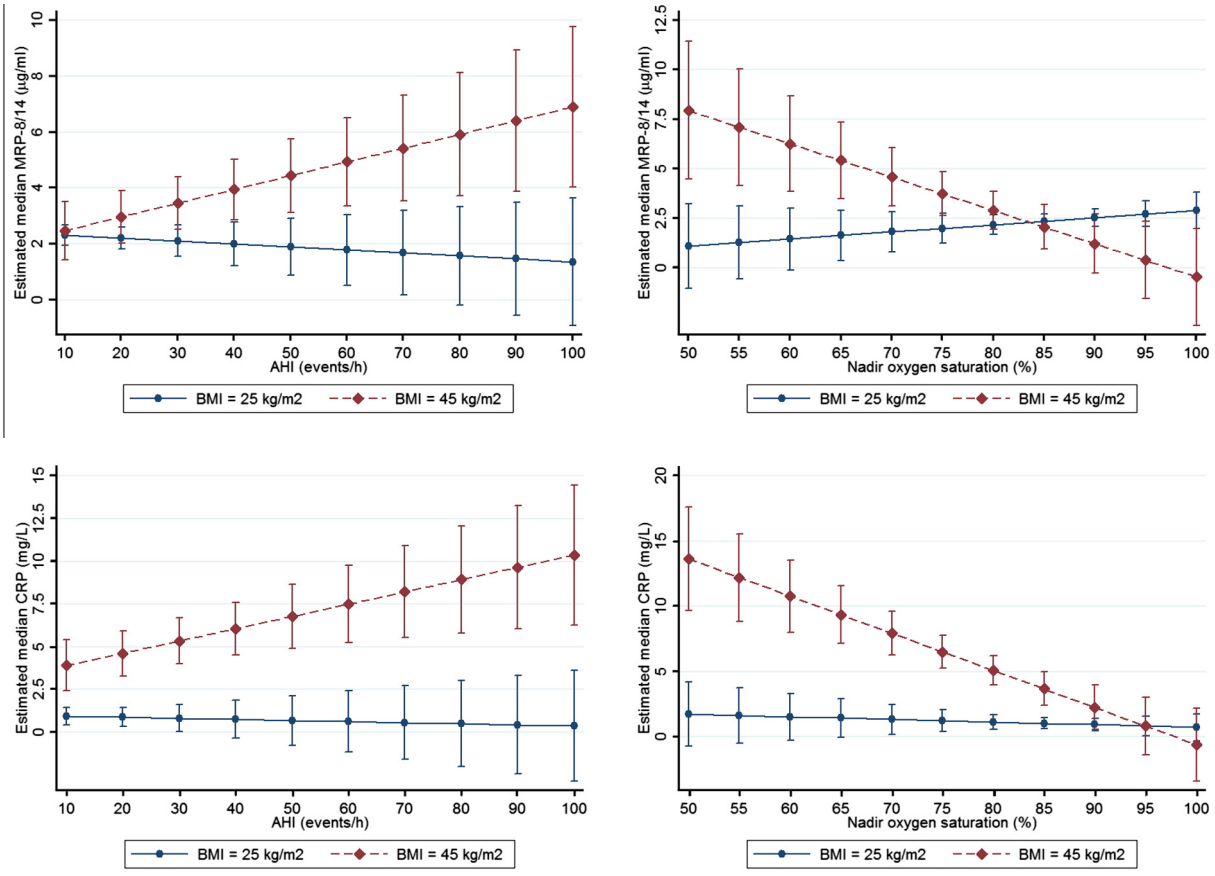

Fig. 1. Median (95% confidence interval) inflammatory biomarker values according to deciles of apnea–hypopnea index (AHI) or vigintiles of nadir oxygen saturation by two examples of body mass index (BMI; 25 and 45 kg/m^2) estimated from multivariate models with interaction terms. The curves diverge due to a statistically significant interaction between BMI and AHI or nadir oxygen saturation on inflammation. MRP-8/14, myeloid-related protein-8/14; CRP, C-reactive protein.

were found between average oxygen saturation and MRP-8/14, and between ODI and CRP. Finally, we observed interactions between BMI and several variables of sleep-disordered breathing on MRP-8/14 and CRP levels.

Repetitive cycles of disrupted ventilation during sleep are the hallmark of OSA. The recurrent bouts of hypoxia, arousal and increased negative intrathoracic pressure represent a conglomerate of noxious stimuli leading to oxidative stress, activation of the sympathetic nervous system, vascular endothelial dysfunction, and mechanical stress on the myocardium and large arteries [3,34]. Several of these processes may promote immune system activation leading to increased levels of inflammatory biomarkers. In line with this, the distribution of CRP was different according to groups of AHI <5 or ⩾5. The same unadjusted comparison revealed no difference for MRP-8/14, but the negative correlation with age renders interpretation difficult. In fact, in baseline models adjusted for age and sex, AHI was associated with both MRP-8/14 and CRP, and it remained a predictor of CRP after adjustment for BMI. However, in final models including additional factors the association between AHI and inflammation did not remain statistically significant. In these models several potential confounders were included [35–37]. Hypertension, impaired glucose metabolism, and cardiovascular complications are prevalent in patients with OSA [2,38]. These factors, in addition to others such as adverse lipid profiles, smoking and poor socio-economic status, have been shown to be associated with inflammation in various populations [35–37,39]. Moreover, inflammation is closely linked to CVD [4,5]. Nevertheless, the specific causal pathways potentially linking apneas and

hypopneas, inflammation, other risk factors, and CVD remain complex and not fully elucidated, and the analyses of our cross-sectional sample must therefore be interpreted with caution. It is also possible that variable population sizes, degree of confounding factors and unequal inclusion and exclusion criteria contributed to a diversity of results in previous studies investigating inflammation in OSA [25–28]. Recently, a large meta-analysis showed elevations of several inflammatory biomarkers in OSA when compared to controls, whereas Xie et al. found evidence in 1985 patients from 35 different studies that treatment with continuous positive airway pressure could partially suppress inflammation [29,30]. These latter analyses underscore that meticulous attention to study size and selection is important in interpreting results. Ultimately, additional large and well-designed trials are needed to establish whether inflammation is directly implicated in the causal pathway between OSA and CVD, or whether it is merely a disease marker.

The associations of various metrics of oxygen saturation to inflammatory biomarker levels differed between MRP-8/14 and CRP. In the final models, average oxygen saturation was an independent predictor of MRP-8/14 levels, whereas ODI was a predictor of CRP. In fact, the latter was the only statistically significant association observed with oxygen variables in the BMI model. These findings support the concept that MRP-8/14 and CRP have different pathways of expression and/or breakdown patterns, which is also suggested by their modest correlation. Intermittent hypoxia has been proposed as a possible trigger of inflammation through nuclear factor κ-B-mediated pathways in patients with OSA [34]. MRP-8/14 is secreted mainly by neutrophils and monocytes, but the underlying mechanisms remain less well understood [40]. CRP has been thought to be synthesized by hepatocytes in response to factors produced by adipocytes and inflammatory cells. Extrahepatic tissues, notably adipocytes and smooth muscle cells, have also been demonstrated to express CRP [23,24]. In view of these observations, it is interesting that this study found a quantitative interaction between BMI and several variables of sleep-disordered breathing on both biomarkers. A recent publication described a possible interaction between obesity and OSA on interleukin-6 and CRP [25]. These findings support the hypothesis that adipocytes may be involved in CRP production, but one cannot exclude that it is an indirect effect promoting increased synthesis from hepatocytes. It is not known why a similar phenomenon was observed for MRP-8/14. Nonetheless, the results from our analyses indicate that individuals with a higher BMI demonstrated an amplified inflammatory response in association with worsening levels of several variables of sleep-disordered breathing.

Our findings have implications for future studies. In unselected individuals evaluated for OSA, an independent effect of apneas and hypopneas on inflammation was not established with statistical significance in final models. Current knowledge indicates that most obstructive apneas and hypopneas cause hypoxia and reoxygenation cycles leading to production of reactive oxygen species. Whether it is the hypoxic or reoxygenation phase, or both, the cause of this phenomenon remains unknown [34]. In theory, this intermittent hypoxia activates pathways to an inflammatory response. Accordingly, ODI retained an independent association with CRP when other factors associated with inflammation were simultaneously considered, and one could speculate that AHI trended in the same direction. It remains debatable whether the factors we included as confounders represent true, separate, independent mediators of inflammation in individuals investigated for OSA, as they may be merely disease markers or downstream effects of inflammation per se. Therefore, we cannot rule out that with a larger and more selected population we might demonstrate that noxious stimuli resulting from apneas and hypopneas are independently associated with CRP. In contrast, only average oxygen saturation was found to possess an independent association with MRP-8/14 in the final models. The demographic profile of the individuals included in our study makes it difficult to compare our findings with previous data from children, in whom most indices of worsening OSA have been found to be independently related to increased MRP-8/14 levels [20,21]. We believe that average oxygen saturation represents hypoxic exposure over time and is more prone to confounding by other causes of altered respiratory function. Also, differing secretion pathways, biological stability, and breakdown mechanisms may have contributed to the disparities observed between MRP-8/14 and CRP.

The present study has some notable strengths and limitations. Current knowledge of MRP-8/14 in adults investigated for OSA is sparse. The inclusion of CRP allowed us to assess the MRP-8/14 values in the context of a well-established marker of inflammation. Comorbidity data were sampled at inclusion and our largely unselected population enhances the external validity of the results. Potential components such as socio-economic status, alcohol consumption, hormone replacement therapy, and menopausal status were not available. Sleep quality, total recording time, and favoured body position may vary between PG and PSG and this could have influenced the results. Also, measures of insulin resistance, advanced glycation end-products, and endothelial function would have complemented and contributed relevant information to our observations, as they may be implicated in relevant causal pathways that were operating in our population. Ultimately, the cross-sectional design makes it inappropriate to propose any causal associations between OSA and inflammation, and we cannot exclude that MRP-8/14 or CRP, or both, are directly involved as mediators in pathways between OSA and many of the other explanatory variables included in our regression models. Addressing these limitations in upcoming trials will be desirable to pinpoint accurately the extent of information provided by inflammatory biomarkers in OSA.

In conclusion, no definitive indication of independent immunological activity resulting from apneas and hypopneas was found in final models adjusted for other factors associated with inflammation, whereas average oxygen saturation for MRP-8/14 and ODI for CRP remained statistically significant predictors. Interactions were observed between BMI and several variables of sleep-disordered breathing on MRP-8/14 and CRP levels.

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Conflict of interest

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: http://dx.doi.org/10.1016/j.sleep.2014.03.008.

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